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Quantitative methods to measure pigmentation variation in farmed Giant Tiger Prawns, *Penaeus monodon*, and the effects of different harvest methods on cooked colour.

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Abstract

Cooked prawn colour is known to be a driver of market price and a visual indicator of product quality for the consumer. Although there is a general understanding that colour variation exists in farmed prawns, there has been no attempt to quantify this variation or identify where this variation is most prevalent. The objectives of this study were threefold: firstly to compare three different quantitative methods to measure prawn colour or pigmentation, two different colorimeters and colour quantification from digital images. Secondly, to quantify the amount of pigmentation variation that exists in farmed prawns within ponds, across ponds and across farms. Lastly, to assess the effects of ice storage or freeze-thawing of raw product prior to cooking. Each method was able to detect quantitative differences in prawn colour, although conversion of image based quantification of prawn colour from RGB to Lab was unreliable. Considerable colour variation was observed between prawns from different ponds and different farms, and this variation potentially affects product value. Different post-harvest methods prior to cooking were also shown to have a

profound detrimental effect on prawn colour. Both long periods of ice storage and freeze thawing of raw product was detrimental to prawn colour. However, ice storage immediately after cooking was shown to be beneficial to prawn colour. Results demonstrated that darker prawn colour was preserved by holding harvested prawns live in chilled seawater, limiting the time between harvesting and cooking, and avoiding long periods of ice storage or freeze thawing of uncooked product.

Keywords:
Shrimp, color, astaxanthin

1 Introduction

Most prawns have thin opaque shells, and colour is present in the hypodermal layer in pigment structures, known as chromatophores (Rao, 1985). These structures are known to expand and contract which strongly contributes to the degree of individual colouration (Fingerman, 1965), particularly in response to the colour of the substrate the animal is exposed to. The colour itself is due to the presence of the carotenoid astaxanthin (Axn) in the hypodermal tissue and the exoskeleton (Katayama et al., 1971). Like all crustaceans, pigmentation in the Black Tiger prawn, *Penaeus monodon*, is known to be produced by the interaction between Axn and a protein called crustacyanin (CRCN) (Zagalsky et al 1985). This interaction turns the colour of Axn from red to blue, but when prawns are cooked this interaction is disrupted, releasing the red colour once again and providing the distinct red colouration of cooked crustaceans. This colour has been shown to be a strong element in consumer preference and acceptance (Erickson et al., 2007; Parisenti et al., 2011), with consistently dark red coloured animals attracting premium prices.

Differences in prawn colouration can be potentially due to a range of factors including carotenoid availability in the diet, background substrate colour, photoperiod, light intensity, stress, temperature or genetics (Rao, 1985; Latscha 1989). Some of these changes are rapid, reversible, rhythmic and under the control of eyestalk hormones (Kleinholz, 1961; Rao, 2001), while others are slower and potentially more permanent, involving modifications of exoskeletal pigment concentration or composition. The best studied effectors of prawn pigmentation have been dietary Axn incorporation and exposure to different coloured substrates. Prawn colouration is dependant largely upon the amount of Axn present within these tissues, with dietary Axn levels of up to 200 mg/kg shown to be most effective for optimal colouration in *P. monodon* (Howell and Matthews 1991; Menasveta et al., 1993; Boonyaratpalin et al., 2001). However, total prawn Axn content does not correlate well with prawn colour (Tume et al., 2009). Short-term exposure to black substrates has also been shown to improve prawn pigmentation through expansion of epithelial chromatophores (Tume et al., 2009; Parisenti et al., 2011a). An increase in the abundance of epithelial CRCN

protein was further demonstrated to be the underlying cause of these pigment improvements (Wade et al., 2012).

Cooked prawn colour is commercially scored by subjective comparison of individuals against either an Australian Tiger Prawn Colour Chart (Aqua Marine Marketing), or international Salmofan colour scale (DSM Nutritional Products). Prawn colour has successfully been quantified using colorimeters (Parisenti et al 2011; Wade et al., 2012). These machines quantify colour using the Commission Internationale de l'Eclairage (CIE) *'Lab'* system of colour notation (Publication CIE No 15, 2004). The absolute colour of a sample is measured on a three dimensional scale of value, hue and chroma. The value of colour (or lightness represented by *'L'*) has a scale of 0 (pure black) to 100 (pure white). The hue has two components that distinguish opposing colours. The first is *'a'* which represents the red-green scale, and the other is *'b'* which represents the blue-yellow scale. Chroma (or saturation) indicates the amount of hue, positive *'a'* towards red, negative *'a'* towards green and positive *'b'* towards yellow, negative *'b'* towards blue. Additionally, the use of digital images to quantify colour in live organisms is common, and has been successfully used to quantify shell pigments in mangrove crabs (Todd et al., 2011) and clawed lobsters (Tlusty and Hyland 2005).

The objectives of this study were firstly to assess three different quantitative methods to measure prawn colour, two different colorimeters and colour quantification from digital images, and the ability to compare colour values from these three methods. Secondly, to use these methods to quantify any variation that exists between the colour of farmed *Penaeus monodon* from different ponds, or from different farms. Lastly, to assess how different types of harvest method, specifically how ice storage and freeze-thawing prior to cooking, affect the colour of farmed *Penaeus monodon*.

2 Material and Methods

2.1 Quantitative and Subjective Measurement of Prawn Colour

Prawn colour was quantified using the average colour of the first three abdominal segments measured using three different methods. The first used a HunterLab Mini Scan XE colorimeter with a 10 mm aperture and D65 illumination at a 45˚ angle. The second used a Minolta CR-400 Chroma Meter with an 8 mm aperture and D65 illumination at a 10˚ angle. The third method used digital images taken at a distance of 40 cm using a Canon D-400 (Canon) fitted with an 18 mm lens, with fixed settings of ISO1600, aperture F22 and 1/100th sec shutter speed. Animals were photographed in a 38 x 50 cm light box illuminated with 2 x 8W 30 cm Fluroglow single reflector full spectrum aquarium lights (AquaOne). Average RGB values were calculated across a 3600 pixel square from the first three abdominal segments using ImageJ software (Schneider et al. 2012). Where necessary, image intensity was adjusted between photographs using the MacBeth ColorChecker that was positioned in each photograph (Supplementary Figure 1A). Subjective scoring was performed against both the Lineal Salmofan (DSM Nutritional Products) and Australian Tiger Prawn Colour Chart (Aquamarine Marketing) under standardised illumination by experienced researchers.

Validation of the digital image method was performed by quantification of the MacBeth colour checker, Salmofan and prawn colour chart values measured from 10 independent photographs (Supplementary Figure 1B, Supplementary Table 1). Comparison of the three different methods was performed using the MacBeth color checker, as well as the colour values quantified from the same randomly selected 45 cooked prawns. Due to the size of the animals, colour quantification for the 45 animals from digital images was performed across three photographs containing 15 animals each. RGB values from digital images were converted to *Lab* values using standard colour conversion algorithms (Nishad and Chezian, 2013) and validated using measurements of the MacBeth color checker from 10 independent photographs (Supplementary Figure 1C).

2.2 Colour Variation Within and Across Ponds and Across Farms

Prawn colour variation was assessed from different ponds from the one farm using a Hunterlab Miniscan XE colorimeter. Fifty prawns were selected at random from holding bins immediately after harvesting from different ponds. The average *Lab* reading from the first 3 abdominal segments was used as the measure of colour for individual prawns. Individuals were tagged, colour measured raw, then cooked in commercial salt brine boilers and re-measured on the cooked prawns. All animals were from domesticated stocks of the same genetic origin, and fed the same commercial diet according to an optimal pigmentation regime that incorporated 50 ppm astaxanthin for at least 4 weeks before harvest and sampling. To assess colour variation between groups, each individual *L*, *a* and *b* colour value was standardised by subtracting the mean value of the entire group. These individual *delta L*, *delta a* and *delta b* values were used to assess the mean and variance for each group of animals. This transformation also allowed effective comparison of measurements performed using different colorimeters despite their difference in absolute colour value.

Comparison of prawn colour was performed from four different farms using a Minolta CR-400 chroma meter. A random sample of 40 cooked animals and measured, having been harvested from a mixture of different ponds and processed at the separate farms on the day of sampling. The average *Lab* reading from the first 3 abdominal segments was used as the measure of colour for individual prawns. Similar to above, to assess colour variation between groups each individual *L*, *a* and *b* colour value was standardised by subtracting the mean value of the entire group. These individual *delta L*, *delta a* and *delta b* values were used to assess the mean and variance for each group of animals.

2.3 Effect of Harvest Method on Colour

To measure the effect of harvesting prawns live in chilled seawater, prawns from the same pond were held live in aerated 12˚C filtered seawater in large covered 800 L bins. Twenty animals were collected immediately after harvesting, individually tagged and colour measured a using a HunterLab Miniscan XE. These same 20 animals were recovered and re-measured at 30 min, 1 hour, 2 hours and 4 hours after the initial measurement. Similarly, to measure

the effect of harvesting prawns into an ice slurry, 20 prawns were individually tagged immediately after harvesting and colour measured a using a HunterLab Miniscan XE. These animals were held in a slurry of ice and filtered seawater and the colour of each one re-measured every hour over an eight-hour period. The change in absolute colour over time for both these groups was calculated by subtracting the average initial *Lab* value from each of the measured *Lab* values of the 20 prawns at each time point. These individual *delta L*, *delta a* and *delta b* values were used for comparison over time.

To assess the effect of freeze-thawing on uncooked prawn colour, 50 prawns were colour measured raw using a HunterLab Miniscan XE, then frozen for one day, thawed at room temperature for 1 hour and colour re-measured. To assess the effect of ice slurry storage on cooked prawn colour, 50 cooked prawns were colour measured using a HunterLab Miniscan XE, then placed in an ice slurry for 14 hours, then colour measured again. The change in absolute colour for these treatments was calculated by subtracting the average initial *Lab* value from each of the measured *Lab* values of the 50 prawns after being re-measured. These individual *delta L*, *delta a* and *delta b* values were used for comparison before and after treatment.

2.4 Statistical Analysis

Where required, statistical significance was assessed by single factor analysis of variance (ANOVA), followed by Tukey's HSD test allowing 5% error. F-Test for significant differences in variance between two groups was performed after Kolmogorov-Smirnov/Lilliefor Test for data normality. All statistical analyses were performed using StatPlus:Mac 2009 (AnalystSoft Inc, 2009).

3 Results

3.1 Quantitative Methods for Measuring Prawn Colour

Absolute *Lab* and RGB values from each of the three methods were obtained from the average of 45 randomly selected animals (Table 1). There was considerable difference between the average absolute *Lab* values measured with the different colorimeters. This was expected given the different light incident angles that the machines have for measurement. We recorded a strong linear relationship between values of the MacBeth color checker measured with each colorimeter (Supplementary Figure 1B). Despite this relationship, a simple linear model was not sufficient to convert *Lab* values from one machine to the *Lab* values of the other (data not shown). In addition, we also observed a strong relationship between the *Lab* values of each machine using individual prawns (Figure 1A).

Using digital images and the MacBeth color checker it was possible to reliably measure colour (Supplementary Figure 1B), and convert RGB values to *Lab* values (Supplementary Figure 1C). Image quantification could also reliably reproduce the colour scales of the Lineal Salmofan (DSM Nutritional Products) and the Australian Tiger Prawn Colour Chart (Aquamarine Marketing), which are the internationally recognised subjective methods for subjectively grading prawn colour (Supplementary Table 1). However, using cooked prawns the conversion of RGB values to *Lab* values did not show any relationship with measured *Lab* values from colorimeters (Figure 1B).

3.2 Colour Variation in Farmed Prawns

It was hypothesised that significant variation existed between the colour of prawns from different ponds. This was found to be true, with farmed prawns showed considerable variation in colour between ponds when either raw or cooked. The mean absolute *Lab* values were significantly different between animals from different ponds, for both their raw colour and their cooked colour (Table 2). For raw prawns, the *L* values were particularly informative. When transformed relative to the average of all samples, data showed that raw prawns from ponds 2 and 3 were significantly higher than those from ponds 1 and 4 (Figure 2A). This result indicated that prawns from ponds 2 and 3 were

234 significantly lighter than those from ponds 1 and 4. The mean *a* and *b* values of
235 cooked prawns were most informative, and significant differences were
236 observed between animals from different ponds (Table 2). When transformed
237 relative to the average of all animals, higher *a* and *b* values indicated the
238 presence of more red and yellow hues, respectively, and therefore a darker
239 coloured prawn. Groups of prawns from different ponds also showed different
240 amounts of colour variation within each group. The variance of *L* and *a* values of
241 cooked colour was significantly higher for some ponds than for others (Table 2),
242 and was reflected by the greater spread of the interquartile range for some
243 ponds (Figure 2B). This indicated there was a greater amount of individual
244 colour variation in some ponds compared with others. Interestingly, *b* values did
245 not show any significant differences in variance between ponds. Some weak
246 correlations were observed between raw *Lab* values and cooked *Lab* values, the
247 best of which was a negative correlation between raw *L* value and cooked *a* value
248 (r^2 = 0.161). This indicated that an increase in *L* value of an uncooked prawn
249 would result in a decrease in the *a* or 'redness' value of the cooked prawn.
250 When comparing cooked prawn colour across farms, similar variation was
251 observed. The absolute *Lab* values of cooked prawns was significantly different
252 between animals from four farms (Figure 3). In some instances, such as farm 1,
253 lower absolute *a* values were recorded, along with higher *L* values, indicating
254 that prawns had a lighter colour with less red. Other farms, such as farm 2,
255 recorded significantly elevated *a* values and slightly reduced *L* values, indicating
256 darker and deeply red coloured prawns.
257 Although the methods differed slightly, some comparison was possible between
258 the absolute *Lab* values recorded for uncooked and cooked *Penaeus monodon* in
259 this study and the *Lab* values recorded for *Penaeus vannamei* (Parisenti et al
260 2011b). Raw *L* readings confirmed that *Pmon* (*L* = 16.02) was a much darker
261 colour than even the most pigmented *Pvan* (*L* = 27.99), and this translated into a
262 lower cooked colour *L* value (*Pmon L* = 40.98; *Pvan L* = 61.49). Uncooked *a* and *b*
263 values showed some small differences, but were all close to zero. However, the
264 cooked *a* or 'redness' value was markedly higher in *Pmon* (*a* = 38.62) than in
265 *Pvan* (*a* = 27.25), while b values were similar (*Pmon b* = 36.04; *Pvan b* = 38.34).

This supports the notion that uncooked *L* values are the best indicator of cooked *a* value, and therefore the deep red cooked colour preferred by consumers.

3.3 Effect of Live Holding in Bins on Colour of Prawns

As a common harvest method, prawns are transferred in large bins from the harvest pond to the processing shed. It was hypothesised that significant variation in prawn colour may occur during this process, which may negatively impact cooked prawn colour. Animals that had been held live in covered and aerated 800 L bins for different periods of time showed very little change in cooked colour after up to 4 hours holding prior to cooking (Figure 4A). Only the *b* value of animals sampled at 30 min and the *L* value of animals sampled at 4 hours were significantly different from the values of animals sampled at other times. Subjective scores showed that animals retained scores of between 9 and 10 on the Prawn Colour Chart, and 29 on the Salmofan throughout holding (data not shown). Although prawns have been shown to rapidly respond to the colour of their surroundings, such as the colour of holding bins (Tume et al., 2009), bins used in this study had lids that completely blocked the light while animals were being held. This method was shown to be highly effective at preserving prawn colour during holding prior to cooking.

3.4 Effect of Ice Storage or Freezing on Colour of Prawns

Although less common, other commercial harvest methods include direct immersion of prawns into an ice slurry, or freezing of raw product. It was hypothesised that these methods were adversely affecting cooked prawn colour. To assess the effect of ice storage prior to cooking, the same 20 prawns were colour measured over time during ice storage. We measured a significant increase in the *L* value after 4 hours while the *a* and *b* values were unaffected (Figure 4B). In a similar experiment, a group of 50 prawns was measured before and after 14 hours of ice storage. Animals after this ice storage period showed a significant increase in their average measured *Lab* values, coupled with a significant increase in *L* variance (Table 3). The effect of freeze thawing was assessed using another 50 prawns measured before being frozen and remeasured once thawed. This treatment also caused a significant increase in

299 each of the measured *Lab* values, and a significant increase in the variance of the
300 measured *L* values (Table 3).
301 After cooking, prawns are held overnight in large bins containing a salt brine ice
302 slurry to improve flavour and storage life, but the effect of this treatment on
303 colouration has not been quantified. To assess the impact of ice-storage after
304 cooking, the same group of 50 prawns was measured immediately after cooking
305 and again after 14 hours in an ice slurry. Results showed there was a small but
306 significant decrease in the measured *L* value of cooked prawns after being held in
307 an ice slurry, along with a significant increase in the *a* and *b* values (Table 3).
308 Variance was not significantly changed in any of the *Lab* values after freeze
309 thawing. This indicated the presence of more red and yellow hues, and
310 demonstrated this treatment was having a positive effect on prawn colour.

4 Discussion

Our results demonstrate that quantitative differences in individual prawn colour can be detected by either colorimeter as well as digital images. However, at present the values measured from prawns using the different techniques cannot be accurately interconverted. Some improvements in the error rate of conversion of RGB values to *Lab* values may be possible using neural network models, instead of linear models such as those used in this study (León et al., 2004). However, the accuracy of conversion of the MacBeth color checker suggests that the errors are perhaps not occurring during conversion. It is far more likely that the inability to convert RGB values from images of prawns to *Lab* values measured from colorimeters is due to the inconsistency of measurement with the smaller aperture of the colorimeter. In the past, the use of colorimeters has been criticised due to the small area represented by the machine, and that aspects of the overall colour are lost (Mendoza and Aguilera, 2004; Papadakis, et al., 2000). This may be particularly evident with the spatial variation in colour across prawn segments, and highlights the importance of establishing a consistent location for colour measurement methods. Given these difficulties, it is not recommended that conversion of colour values be performed from images to colorimeters, but data from different colorimeters can potentially be compared. Although images were not extensively used in this study, they represent an inexpensive, rapid and accurate method for assessing prawn colour.

This study quantified the variation that existed in farmed prawns, and demonstrated that there are significant colour differences both between farms and more interestingly between ponds at the same farm. Some of the observed variation may be due to a range of farm specific conditions, such as different pigmentation regimes in feeds, lined or earthen ponds or different pond algal densities. Carotenoid inputs from pelleted feeds were consistent across ponds measured (50 mg/kg), although differences in the total amount of feed intake for different ponds cannot be accounted for. Although not measured specifically in this study, a large amount of variation has been shown to exist in the phytoplankton, algal and bacterial populations of different prawn ponds (Burford 1997, Xiong et al., 2014). While the diversity of species was similar

344 across ponds, the abundance of species varied markedly and rapidly, often
345 relative to the amounts nutrients available in the water (Burford 1997, Xiong et
346 al., 2014). Potential effects of this pond to pond variation on pigmentation
347 include different levels of cyanobacteria capable of producing carotenoids that in
348 turn affect carotenoid intake. In addition, variation in pond dynamics can
349 potentially affect two other known effectors of crustacean colour: light intensity
350 (Pan et al., 2001) and background substrate colour (Tume et al., 2012). *Penaeus*
351 *mondon* postlarvae cultured under constant light conditions recorded a higher
352 total Axn concentration than those in constant darkness, and this effect was
353 attributed to increased production and accumulation of Axn in algae within the
354 tank that was in turn ingested by the animals (Pan et al., 2001). Prawn colour
355 was also shown to be rapidly darkened by exposure to dark coloured
356 background substrates, but there was no change in Axn concentration (Tume et
357 al., 2009). Potential variation in the colour of pond substrates could not be
358 quantified in this study, but this may influence the colour of the final cooked
359 product. Other reports of the effect of harvest stress on pigmentation are largely
360 anecdotal, with no scientific methods employed to specifically investigate any
361 potential effect. Given the colour variation measured from individual ponds from
362 one farm, the quantified colour variation across farms was more likely due to the
363 variation produced by the conditions within a particular pond at the time of
364 harvesting. Identifying the true source of the measured variation in prawn colour
365 was beyond the scope of this project, and would require a much more detailed
366 study with few additional benefits to the current study.
367 Although it was not possible to predict the precise effect on cooked colour from
368 the measured raw *Lab* values, it was possible to infer the effect from on cooked
369 colour from the negative correlation recorded earlier between raw *L* value and
370 cooked *a* value. This demonstrated that prawns that recorded a higher raw *L*
371 values were not only lighter in colour before cooking, but would record lower *a*
372 values when cooked and were therefore less pigmented. By measuring the same
373 prawns at different times and through different treatments, this study eliminated
374 the variability that had been recorded between individual prawns. Results
375 showed that uncooked prawns that were either held on ice for periods longer
376 than 4 hours or frozen and then thawed became significantly paler in colour. The

effect of freeze thawing raw product was a similar magnitude to that seen over 8 hours of ice storage, and would result in a less pigmented product, a lower colour grade score and corresponding lower price. Very few studies have been done in this area. Flavour has been enchanced in *Macrobrachium rosenbergii* by post-harvest salt acclimation (Schilling et al., 2013), but any potential effects of ice storage on colour were not assessed. Despite improving flavour, this study shows that perceived quality may be adversely affected due to such pre-cooking treatments. This finding may also be relevant for the holding of prawns during wild fisheries operations. Although impractical to immediately cook prawns at time of harvest, the method by which they are stored on board the trawler may significantly impact product quality. Prior to the development of accurate and unbiased methods in this study, and the ability to correlate raw prawn colour with cooked colour, the effects of different harvest methods could not be quantified.

Once cooked, prawns are often preserved in salted ice slurry overnight to improve flavour and shelf-life in storage. However, the effect of this treatment on colour has not been quantified in the past. This study demonstrated that post-cooking storage in an ice slurry was having minimal, if not a slightly beneficial, effect on prawn colour. Cooking time has been shown to affect the appearance of dark spots during frozen storage of *Penaeus vannamei* (Manheem et al., 2013), but the effect on absolute colour was not assessed. The ability to preserve this cooked colour during frozen storage under commercial conditions is currently under further investigation.

The focus of this study was to identify and quantify whether any variation existed in pigmentation under different commercial conditions and from commercial different farms. What is evident is that the differences in perceived quality, and therefore price, are affected by conditions during commercial grow-out and harvesting. Based on the results of this study, it is recommended that prawns be held live during harvest prior to cooking and processed as quickly as possible after harvest. Salt brining post cooking is beneficial to both colour and flavour. The information from these studies provides industry a sound basis for product handling decisions during processing of prawns to retain maximum red colouration of product.

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7 Figure Legends

Figure 1. Comparison of quantitative methods of measuring prawn colour. The colour of the same 45 cooked prawns was quantified using two different colorimeters and also digital images. Comparison of the absolute *Lab* values taken using a Minolta CR-400 Chroma Meter and a HunterLab Miniscan XE colorimeter (A). Comparison of the absolute *Lab* values taken using a HunterLab Miniscan XE colorimeter and the absolute RGB values quantified from digital images that had been converted to *Lab* values using standard algorithms (B).

Figure 2. Colour Variation in Farmed Prawns Across Ponds. The median (square) and Q1-Q3 interquartile range (bars) distribution of delta *Lab* values from fifty prawns from seven different ponds when uncooked (A) and cooked (B). The delta *Lab* values were calculated as the difference in the value of each individual from the average of all the animals across the 7 ponds. Significant differences in mean and variance between groups are shown in Table 2.

Figure 3. Colour Variation in Farmed Prawns Across Farms. The median (square) and Q1-Q3 interquartile range (bars) distribution of delta *Lab* values from forty cooked prawns from a further four different farms measured using a Minolta Chroma Meter. The delta *Lab* values were calculated as the difference in the value of each individual from the average of all the animals across the four farms.

Figure 4. Colour change in uncooked prawns over time. Prawns harvested from the same pond were held in large 800 L aerated bins that contained seawater at 12˚C. Twenty animals were taken at random immediately after harvesting and after being held in the bins for different lengths of time. Animals were cooked and then quantitatively colour measured using a HunterLab colorimeter. Results are shown as delta *Lab*, which is the difference in absolute *Lab* colour value at each time point relative to the initial sample. * denote significant ($P<0.05$) differences in *Lab* value from the initial measurement.

Supplementary Figure 1. Validation of Colour Quantification from Digital Images. Photos were taken under standardized light and camera settings, and each included the Prawn Colour Chart, Salmofan and MacBeth colour checker array as colour references. Quantification of prawn colour of each individual was performed using an average of 3 equally sized squares located on the first three abdominal segments as shown by the numbers on one of the animals (A). Correlation between the measured *Lab* values from the Hunterlab Miniscan XE and the Minolta CR-400 chroma meter (B). Correlation between expected *Lab* values from the MacBeth colour checker and the converted *Lab* values from the average measured RGB values of the same squares quantified from ten digital photographs (C).

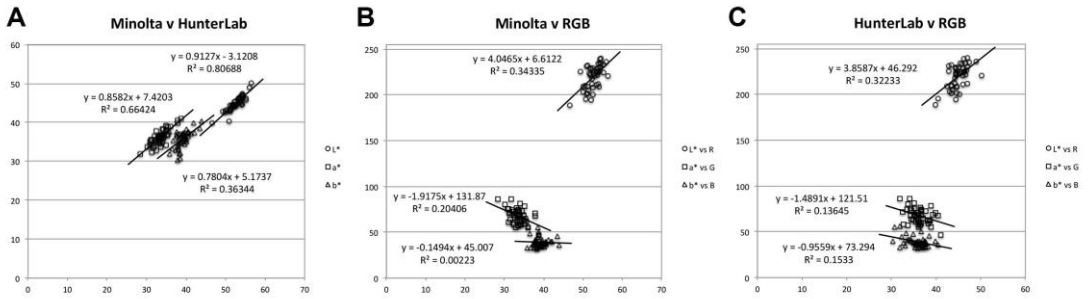


Figure 1. Comparison of quantitative methods of measuring prawn colour. The colour of the same 45 cooked prawns was quantified using 2 different colorimeters and also digital images. **A.** Comparison of the absolute *Lab* values taken using a Minolta CR-400 Chroma Meter and a HunterLab Miniscan XE colorimeter. **B.** Comparison of the absolute *Lab* values taken using a Minolta CR-400 and the absolute RGB values quantified from digital images. **C.** Comparison of the absolute *Lab* values taken using a HunterLab Miniscan XE colorimeter and the absolute RGB values quantified from digital images.

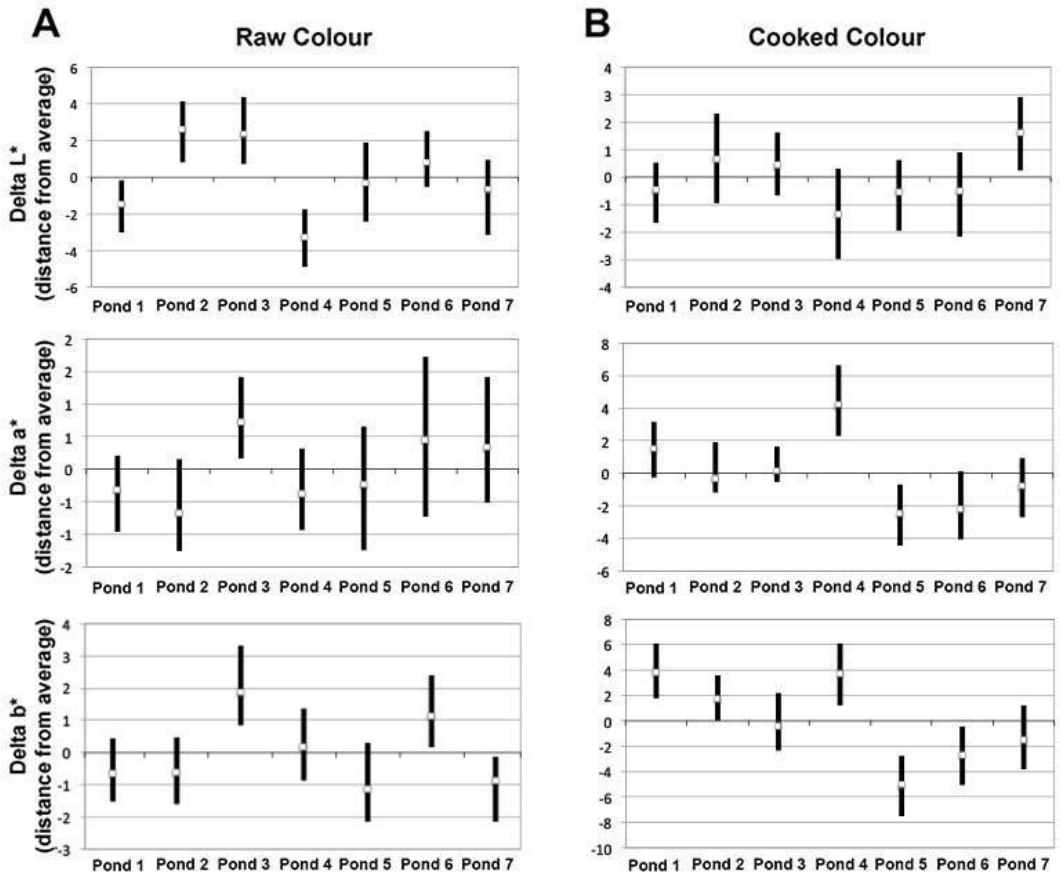
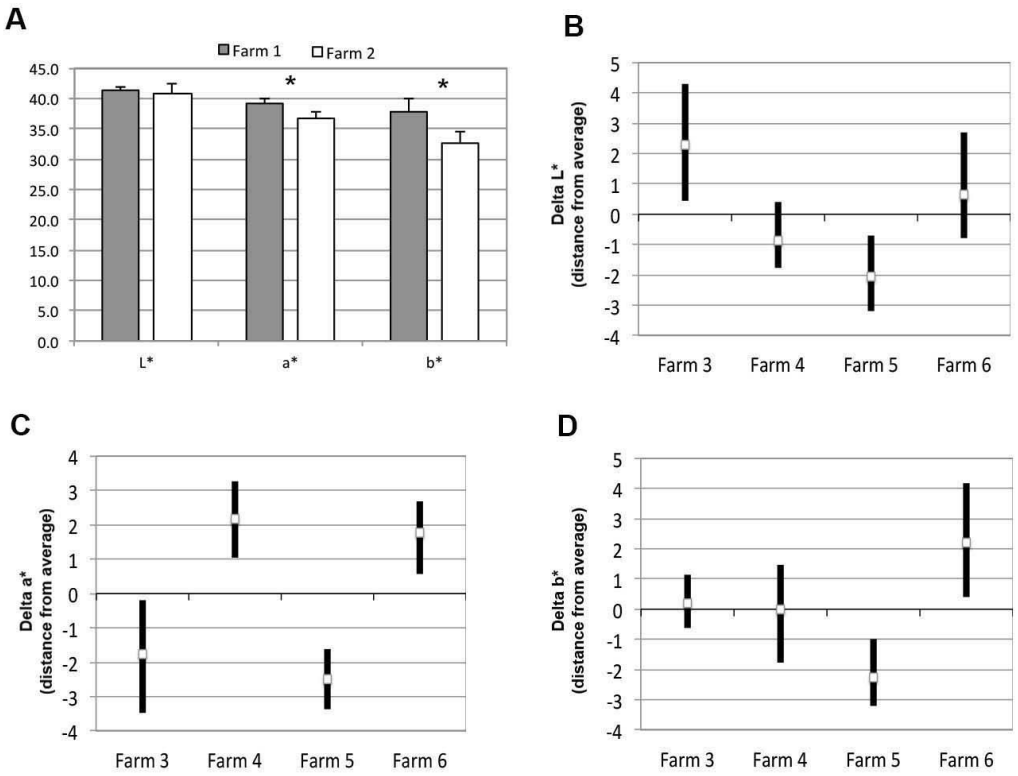


Figure 2. Colour Variation in Farmed Prawns Across Ponds. The median (square) and Q1-Q3 interquartile range (bars) distribution of delta *Lab* values from fifty uncooked prawns from 7 different ponds when uncooked (A) and cooked (B). The delta *Lab* values were calculated as the difference in the value of each individual from the average of all the animals across the 7 ponds.



559
560 **Figure 3. Colour Variation in Farmed Prawns Across Farms. A.** The absolute Lab values for
561 farms 1 and 2 were taken from 3 sets of 50 animals and measured using a HunterLab
562 colorimeter. **B-D.** The median (square) and Q1-Q3 interquartile range (bars) distribution of delta
563 *Lab* values from forty cooked prawns from 6 different farms measured using a Minolta Chroma
564 Meter. The delta *Lab* values were calculated as the difference in the value of each individual from
565 the average of all the animals across the 4 farms. * denote significant ($P<0.05$) differences in *Lab*
566 value between farms.
567

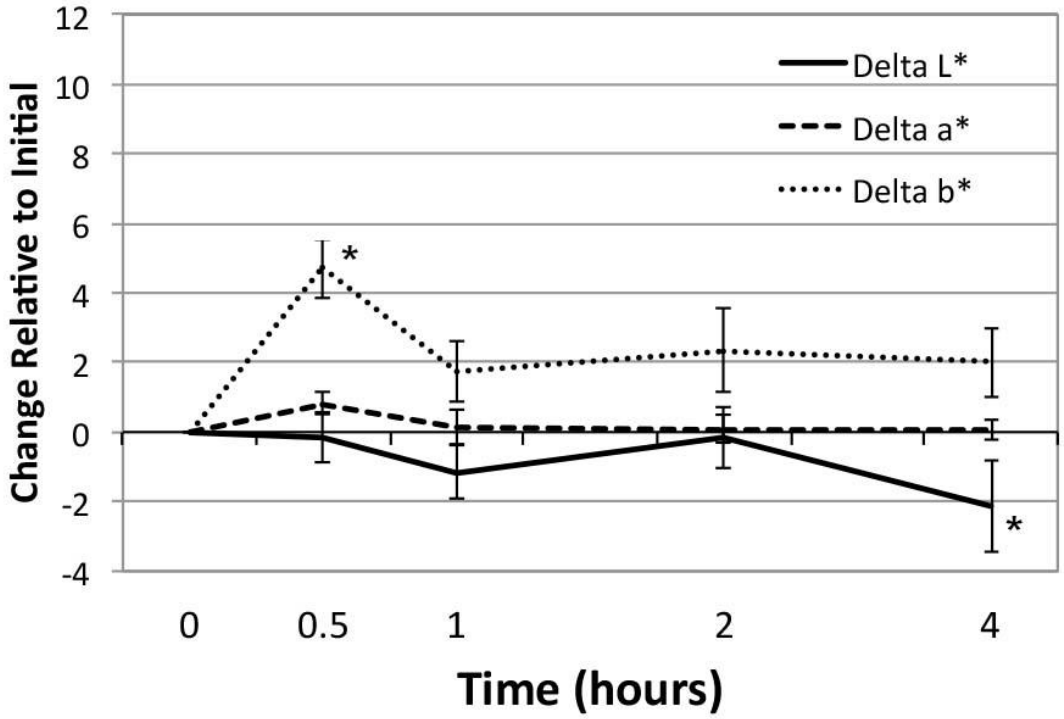


Figure 4. Colour change in uncooked prawns held live in chilled seawater. Prawns harvested from the same pond were held in large 800L aerated bins that contained seawater at 12˚C. Twenty animals were taken at random immediately after harvesting and after being held in the bins for different lengths of time. Animals were cooked and then quantitatively colour measured using a HunterLab colorimeter. Results are shown as delta *Lab*, which is the difference in absolute *Lab* colour value at each time point relative to the initial sample. * denote significant ($P<0.05$) differences in *Lab* value from the initial measurement.

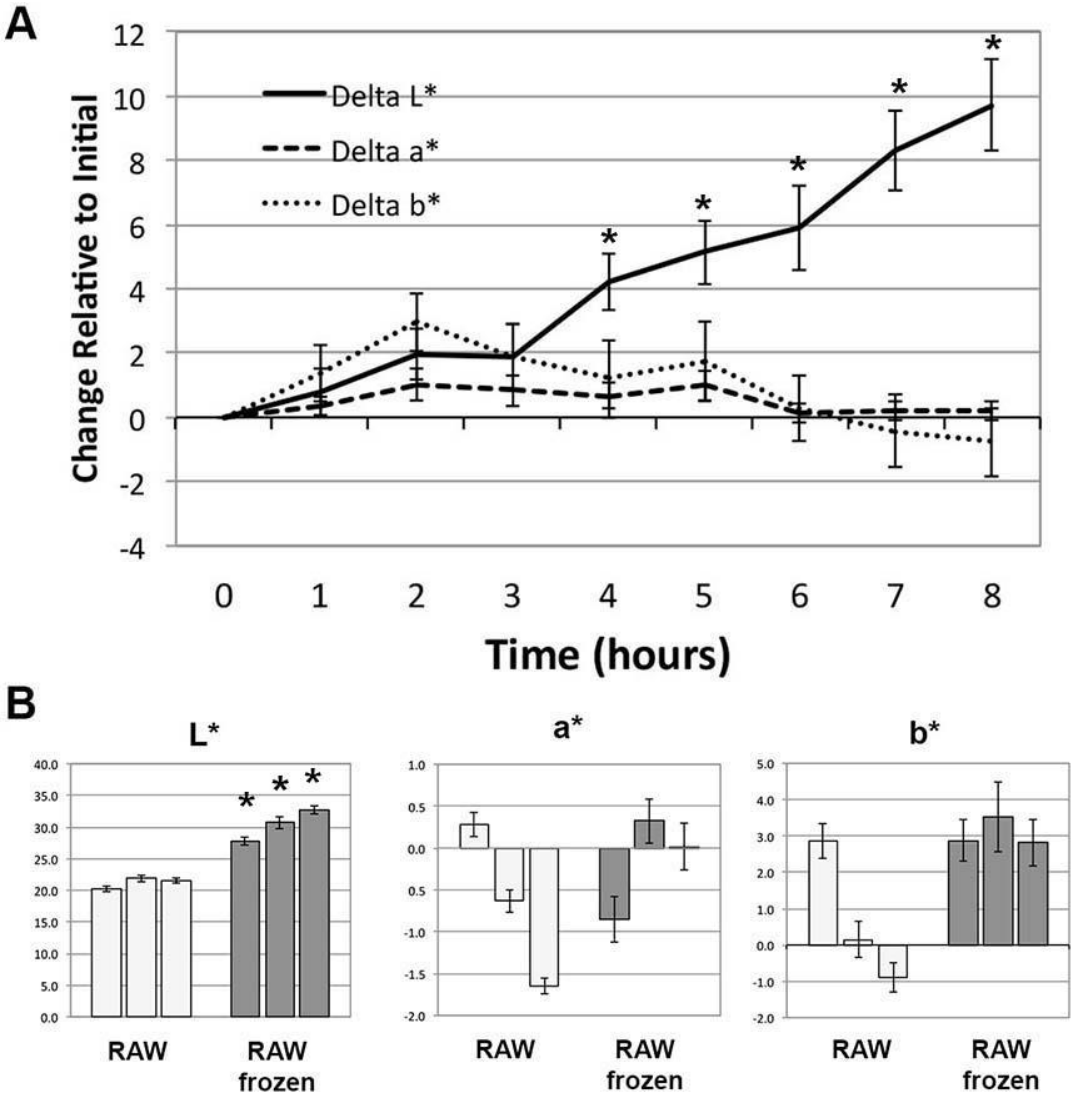
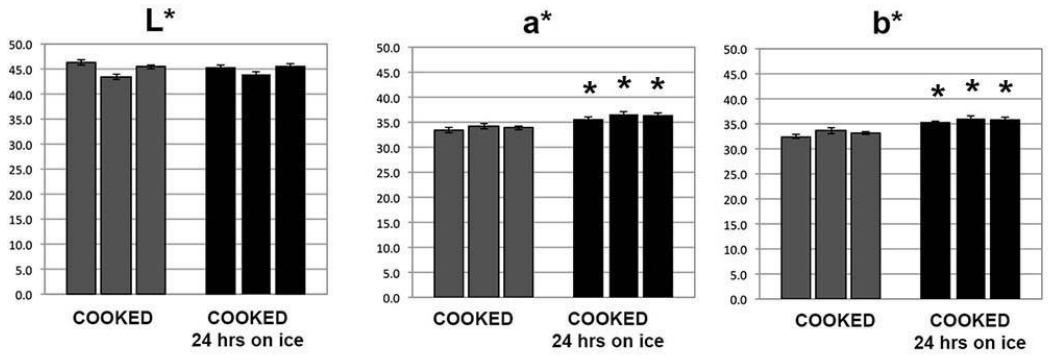


Figure 5. Colour change in uncooked prawns stored on ice or frozen. A. The average *Lab* values were recorded at one hour intervals over 8 hours for twenty uncooked prawns that were stored in an ice slurry. **B.** Each bar represents the average *Lab* colour readings taken from 15 uncooked individuals across the first three prawn abdominal segments. The same animals were measured before and after being frozen and then thawed. * denote significant ($P<0.05$) differences in *Lab* value from the initial measurement.



586
587 **Figure 6. Colour change in cooked prawns after 24 hours ice storage.** Each bar represents
588 the average Lab colour readings taken from 15 cooked individuals across the first three prawn
589 abdominal segments. The same animals were measured before and after 14 hours of storage in
590 an ice slurry. * denote significant ($P<0.05$) differences in *Lab* value from the initial measurement.
591
592

593 Table 1. Mean *Lab* values and variance of colour quantified from farmed Giant
594 Tiger Prawns *Penaeus monodon*.

	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7
Mean							
L*	40.51^{a}	41.64^{b}	41.57^{c}	39.63^{a}	40.44^{a}	40.49^{a}	42.62^{d}
a*	40.10^{a}	38.32^{bcg}	38.74^{bcg}	42.80^{d}	36.13^{ef}	36.42^{ef}	37.84^{g}
b*	40.00^{ad}	37.72^{b}	35.68^{cg}	40.29^{ad}	30.88^{e}	33.18^{fg}	34.55^{cfg}
Variance							
L*	2.34^{a}	5.40^{b}	2.34^{c}	4.81^{d}	4.58^{cd}	8.35^{ce}	5.22^{bcde}
a*	6.13^{a}	10.87^{b}	6.00^{ac}	8.34^{ab}	10.41^{b}	10.11^{b}	7.18^{abc}
b*	10.68	9.08	9.35	13.41	11.48	11.69	9.65

595 Superscripts denote significant ($P<0.05$) differences between measured values.
596

Supplementary Table 1. Validation of image based quantification. Absolute RGB values for each MacBeth Colorchecker square compared with the values quantified from 10 independent photos. The combined RGB values produced the corresponding colours as shown in the table.

MacBeth ColorChecker	Expected Values				Measured Values			
	R	G	B		R	G	B	
dark skin	115	82	68		92 ± 1.33	58 ± 1.31	51 ± 1.39	
light skin	194	150	130		177 ± 1.29	123 ± 1.49	110 ± 1.29	
blue sky	98	122	157		87 ± 1.33	105 ± 1.41	160 ±1.70	
foliage	87	108	67		83 ± 2.31	101 ± 1.34	75 ± 2.23	
blue flower	133	128	177		134 ± 1.85	125 ± 1.92	182 ± 1.27	
bluish green	103	189	170		91 ± 2.98	176 ± 1.60	186 ± 1.68	
orange	214	126	44		181 ± 1.35	81 ± 2.47	38 ± 2.89	
purplish blue	80	91	166		70 ± 1.93	71 ± 1.75	141 ± 1.28	
moderate red	193	90	99		186 ± 1.60	73 ± 1.72	80 ± 1.50	
purple	94	60	108		88 ± 0.85	56 ± 0.56	107 ± 0.96	
yellow green	157	188	64		141 ± 0.95	182 ± 1.74	71 ± 2.21	
orange yellow	224	163	46		225 ± 1.74	146 ± 1.46	59 ± 1.50	
blue	56	61	150		42 ± 1.76	49 ± 1.53	120 ± 1.45	
green	70	148	73		40 ± 1.30	122 ± 1.50	74 ± 1.64	
red	175	54	60		183 ± 1.39	54 ± 1.99	53 ± 1.75	
yellow	231	199	31		221 ± 1.91	187 ± 1.87	53 ± 2.51	
magenta	187	86	149		199 ± 1.08	85 ± 1.87	142 ± 1.74	
cyan	8	133	161		36 ± 1.96	114 ± 1.67	173 ± 1.84	
white	243	243	242		238 ± 1.58	239 ± 1.78	230 ± 1.96	
neutral 8	200	200	200		204 ± 1.64	208 ± 2.20	200 ± 1.89	
neutral 6.5	160	160	160		159 ± 1.51	161 ± 1.92	161 ± 1.88	
neutral 5	122	122	121		125 ± 1.59	123 ± 2.03	125 ± 2.00	
neutral 3.5	85	85	85		89 ± 3.02	83 ± 3.48	92 ± 3.49	
black	52	52	52		51 ± 2.80	48 ± 3.21	51 ± 3.34	

Supplementary Table 2. Quantification using digital images of colour grade charts used for subjective prawn colour grade scoring. The average RGB colour of an equal sized square was quantified across 10 individual photos. The combined RGB values produced the corresponding colours as shown in the table.

	Average			
Salmofan	R	G	B	
20	251 ± 0.79	148 ± 3.45	83 ± 2.77	
21	254 ± 0.28	132 ± 2.45	76 ± 1.94	
22	254 ± 0.34	118 ± 2.19	62 ± 1.56	
23	251 ± 0.89	105 ± 2.00	57 ± 1.33	
24	250 ± 0.93	92 ± 2.23	48 ± 1.46	
25	252 ± 0.72	84 ± 1.99	41 ± 1.29	
26	249 ± 0.95	75 ± 1.42	34 ± 1.08	
27	252 ± 0.66	70 ± 1.80	28 ± 1.07	
28	245 ± 1.79	58 ± 2.01	23 ± 1.02	
29	247 ± 1.73	53 ± 2.20	21 ± 1.20	
30	239 ± 2.43	45 ± 2.10	21 ± 1.47	
31	235 ± 2.49	42 ± 1.65	20 ± 1.41	
32	233 ± 2.27	43 ± 1.58	23 ± 1.33	
33	227 ± 2.59	37 ± 1.73	18 ± 1.40	
34	195 ± 2.74	26 ± 1.88	18 ± 1.77	
	Prawn Colour Chart			
	R	G	B	
PCC 7	244 ± 1.93	118 ± 1.64	51 ± 1.61	
PCC 8	245 ± 1.56	113 ± 3.19	60 ± 2.51	
PCC 9	245 ± 1.46	99 ± 2.57	58 ± 2.00	
PCC 10	246 ± 1.30	78 ± 1.81	45 ± 1.33	
PCC 11	244 ± 1.65	66 ± 2.48	44 ± 1.98	
PCC 12	242 ± 1.92	58 ± 1.73	44 ± 1.46	